

Chs B19

21. A pair of oligonucleotide primers for amplifying a portion of the human INGAP coding sequence, wherein said 'portion' excludes the nucleotides encoding the signal peptide, wherein each of said oligonucleotide primers hybridizes to an opposite strand of a double-stranded INGAP template, wherein a first of said oligonucleotide primers hybridizes to the 5' end of the coding sequence for mature human INGAP and the second of said oligonucleotide primers hybridizes to the 3' end of the nucleotide sequence encoding mature human INGAP.

Chris Bzoch

23. A method of forming an expression construct for producing INGAP in a recombinant host cell,

comprising the step of:

linking a transcription initiation site, a translation initiation site, and a coding sequence for mature human INGAP, to form an expression construct which is devoid of the signal sequence of the coding sequence of INGAP.

24. The method of claim 23 further comprising linking to said coding sequence for mature human INGAP a coding sequence for a histidine tag.

25. The method of claim 23 wherein the transcription initiation site is inducible.

26. The method of claim 25 wherein the transcription initiation site is selected from the group consisting of the *lac* promoter/operator, the *tac* promoter, the *trp* promoter, the *lcl* promoter, and the *tet* promoter.

27. The method of claim 23 wherein the coding sequence for mature human INGAP is obtained by amplification of a "portion" of the human INGAP coding sequence, wherein said portion excludes the nucleotides encoding the signal peptide.

28. The method of claim 27 wherein the

008060" 62E65950

Chris B21

amplification is performed using primers having sequences as shown in SEQ ID NO: 2 and SEQ ID NO: 3.

29. A recombinant construct for expression of Islet Neogenesis Associated Protein (INGAP) activity comprising:

a first nucleotide sequence encoding mature human INGAP, said first nucleotide sequence being operably linked to a transcriptional initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide according to SEQ ID NO: 5 is not present immediately 5' of said first nucleotide sequence.

30. The construct of claim 29 wherein nucleotides 1-16 of SEQ ID NO: 1 are not present 5' of said first nucleotide sequence.

31. The construct of claim 29 further comprising a third nucleotide sequence encoding a histidine tag.

32. The construct of claim 29 wherein the third nucleotide sequence is immediately 5' or 3' to said first nucleotide sequence.

33. The construct of claim 29 wherein the transcriptional initiation site is inducible.

As B221

008060-62E65950

42. The construct of claim 33 wherein the

transcriptional initiation site is the *trp* promoter.

43. The construct of claim 33 wherein the transcriptional initiation site is the *tac* promoter.

44. The construct of claim 33 wherein the transcriptional initiation site is the *tet* promoter.

45. A method of producing biologically active Islet Neogenesis Associated Protein (INGAP) protein from a recombinant host cell comprising the steps of:

culturing a host cell comprising a recombinant construct comprising a first nucleotide sequence encoding mature human INGAP operably linked to a transcriptional initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide according to SEQ ID NO: 5 is not present immediately 5' of said first nucleotide sequence; and

recovering protein from said cultured host cell.

46. The method of claim 45 wherein the construct further comprises a third nucleotide sequence encoding a histidine tag, and INGAP protein is purified using a nickel affinity matrix.

47. A host cell comprising a recombinant construct comprising a first nucleotide sequence encoding mature human INGAP operably linked to a

008060" 62E6960



transcriptional initiation site and a translational initiation site, wherein a second nucleotide sequence encoding a signal peptide according to SEQ ID NO: 5 is not present immediately 5' of said first nucleotide sequence.

Chs a1

008060-62E65960